Photobiomodulation, Photomedicine, and Laser Surgery
Volume XX, Number XX, 2019
© Mary Ann Liebert, Inc.
Pp. 1–10
DOI: 10.1089/photob.2018.4514

Original Research

The Effects of Ultraviolet Light-Emitting Diodes with Different Wavelengths on Periodontopathic Bacteria *In Vitro*

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Abstract

Objective: The aim of this study was to examine effects of recently developed ultraviolet light-emitting diodes (UV LEDs) wavelengths on *in vitro* growth and gene expression of cultural periodontopathic bacteria, and on viability of experimental gingival fibroblasts.
Materials and methods: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus oralis* were irradiated by UV LEDs (265, 285, 310, 365, and 448 nm) at 600 mJ/cm^2 and grown anaerobically *in vitro*. The colony forming units were counted after 1 week. Cell morphology was observed using a scanning electron microscope (SEM). Quantitative real-time polymerase chain reaction was performed to investigate gene expression changes by 310 nm irradiation. Viability of the irradiated human gingival fibroblasts was evaluated using WST-8 assay.
Results: Both 265 and 285 nm resulted in the complete death of bacteria and fibroblasts, whereas 310 nm caused partial killing and suppression of bacterial growth and much less damage to the fibroblasts *in vitro*. Both 365 and 448 nm resulted in no significant change. SEM showed that *P. gingivalis* cells gradually degraded from day 2 or 3 and were severely destructed on day 5 for 265, 285, and 310 nm. The 310 nm irradiation transiently suppressed the transcripts of SOS response- and cell division-relative genes.
Conclusions: Both 265 and 285 nm may induce powerful bactericidal effects and severe fibroblast phototoxicity, and 310 nm may induce partial killing or growth suppression of bacterial cells with much less fibroblast phototoxicity. UV lights may have potential for bacterial suppression, with situations dependent on wavelength, in periodontal and peri-implant therapy.

Keywords: UV LED, periodontopathic bacteria, human gingival fibroblasts

Introduction

Periodontal diseases represent inflammation around the tooth-supporting periodontal tissues that is mainly initiated by chronic infections of periodontopathic bacteria.[1–3] The purpose of periodontal therapy is to arrest infection and inflammation of periodontal tissues.[3,4] However, conventional periodontal therapies, such as mechanical therapy and chemotherapy, do not completely eradicate bacteria.[5,6] Antibiotic therapy also has various disadvantages such as allergy, difficulty in maintaining therapeutic concentrations in periodontal pockets, and occurrence of bacterial resistance.[7–9]

Lasers and photodynamic therapy are beneficial in bacterial elimination, without some of the above limitations.[10,11] Recent studies have suggested the clinical efficacy of laser therapy as an adjunct to scaling and root planing, and it has been reported to produce improved clinical outcomes in the treatment of periodontitis.[12–15] However, laser devices are costly, and cause thermal denaturation and carbonization of soft and hard tissues.[16] Antimicrobial photodynamic therapy (a-PDT) using a combination of light and photosensitizer has also been employed for the treatment of infectious diseases including periodontitis.[17,18] On the other hand, ultraviolet light (UV) irradiation alone has been demonstrated to possess

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bactericidal effects and has been employed for sterilization in medicine.[19,20]

UV irradiation (wavelength: 100–400 nm), an electromagnetic irradiation, can be subdivided into UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm).[21] Recently, UV irradiation has been used for treating skin diseases such as psoriasis and atopic dermatitis, especially UVB around 310 nm, which has a more beneficial therapeutic effect and less side effects for human cells compared with other UV wavelengths.[22–24] The therapeutic effects of UV wavelengths may also be applicable in the treatment of periodontitis and peri-implantitis. However, little is known about the effects of UV wavelengths on periodontopathic bacteria and periodontal tissues[25,26] and only one previous *in vitro* study described the effects of UV light-emitting diodes (LEDs) on oral bacteria.[26] Additionally, compact, portable, easy-to-use UV LED devices are being developed as convenient, cost-effective, light delivery methods for clinical use.[26] Therefore, UV LEDs may be considered for clinical application in periodontal and peri-implant treatment.

We hypothesized that antibacterial effects of UV LEDs would vary greatly depending on the wavelengths. Also, we wanted to evaluate the influence of UV LEDs on periodontal tissues in view of their possible application in periodontal therapy. Thus, the aim of this study was to examine effects of different UV LED wavelengths on cultured periodontopathic bacteria in terms of *in vitro* bacterial growth, cell morphology, gene expressions, and on the viability of experimentally prepared human gingival fibroblasts (HGF-1).

Materials and Methods

LED apparatus

An LED apparatus with UV light 265, 285, 310, 365, and visible blue light 448 nm (Nikkiso Giken Co., Ltd., Ishikawa, Japan) was employed. The apparatus was fixed onto a stand so that the light-emitting end was positioned to match the top entrance of a well (6.8 mm in diameter, 0.32 cm^2) in a 96-well flat-bottom titer plate (Falcon®; Corning Inc., Corning, NY) during irradiation (Fig. 1). Irradiation was performed for ~1 min at a total energy density (ED) of ~600 mJ/cm^2. ED was adjusted by selecting a specific power density, and suitable irradiation time, for each wavelength [598.5 mJ/cm^2 (9.50 mW/cm^2 and 63 s) for 265 nm; 601.0 mJ/cm^2 (9.39 mW/cm^2 and 64 s) for 285 nm; 599.4 mJ/cm^2 (9.99 mW/cm^2 and 60 s) for 310 nm; 602.1 mJ/cm^2 (9.87 mW/cm^2 and 61 s) for 365 nm; and 602.3 mJ/cm^2 (9.56 mW/cm^2 and 63 s) for 448 nm], as shown in Table 1.

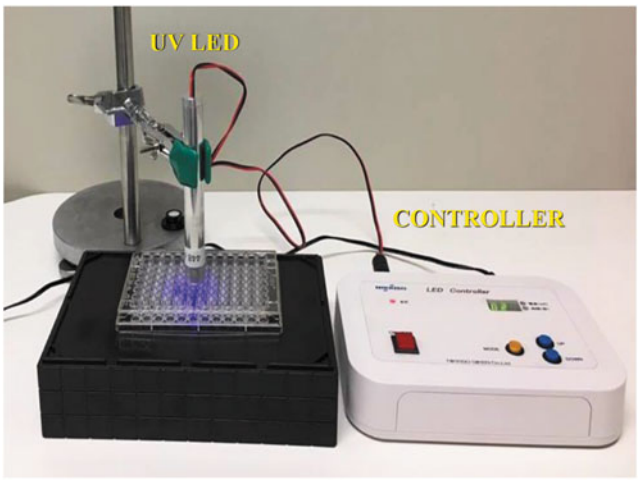

FIG. 1. A UV LED and a power controller. UV LED, ultraviolet light-emitting diode.

Bacteria and culture conditions

Porphyromonas gingivalis ATCC 33277 and *Prevotella intermedia* ATCC 25611 were grown in brain heart infusion (BHI; Difco Laboratories, Franklin Lakes, NJ) and on the agar plate supplemented with 5% (v/v) horse blood as previously described.[27] *Fusobacterium nucleatum* ATCC 25586, *Aggregatibacter actinomycetemcomitans* ATCC 43718, and *Streptococcus oralis* OMZ 607 were grown in BHI broth and on the agar plate supplemented with 5% or 10% (v/v) horse blood, respectively. All bacteria were incubated anaerobically at 37°C.

Preparation of bacterial suspension before LED irradiation

After washing with phosphate-buffered saline (PBS), bacterial cells were adjusted for optical density at 600 nm (OD_{600}) to 1.0 with PBS (~1×10^8 cells/mL) using an Eppendorf Biophotometer™ (Eppendorf AG, Hamburg, Germany). The bacterial cell suspension (100 μL) was added into a well of a 96-well microtiter plate and irradiated by UV LEDs aerobically and anaerobically. After irradiation, the cell suspension was transferred to a 1.5 mL tube, centrifuged, and the supernatant was removed. The resulting bacterial pellet was resuspended with new bacterial culture medium.

Colony forming units of various periodontopathic bacteria

P. gingivalis, *P. intermedia*, *F. nucleatum*, *A. actinomycetemcomitans*, or *S. oralis* suspension was irradiated by UV LEDs aerobically. Irradiated samples were spread on agar plates in triplicate, and incubated anaerobically 1 week at 37°C. Colony forming units (CFUs) were then calculated. The experiment was independently performed four times.

Before and immediately after UV irradiation, the *P. gingivalis* suspension temperature was measured at the center of the suspension at room temperature with a thermocouple device (Digital Thermometer IT-2000; Iuchi, Seieido, Osaka, Japan; $n = 10$).

In vitro P. gingivalis *growth*

P. gingivalis cells were irradiated with UV LEDs anaerobically or aerobically and the OD_{600} was measured at different time intervals up to 24 h ($n = 3$).

Scanning electron microscope analysis

Irradiated *P. gingivalis* was incubated anaerobically for 30 min, 1, 2, 3, and 5 days and fixed in 2.5% (v/v) glutaraldehyde for 2 h at 4°C. After washing three times with 0.1 M phosphate buffer (pH 7.2), they were postfixed in 1% osmium tetroxide for 1 h, dehydrated in ethanol solutions for

Table 1. Ultraviolet Light Irradiation Parameters

Wavelength (nm)	*Power output (mW) 96 well area (0.32 cm²)*	*Irradiation time (seconds)*	*Total energy dose (~600 mJ/cm²)*	*Irradiation spot (mm)*	*Irradiation distance (mm)*
265	9.50 mW	63	598.5 mJ/cm²	6.8	7
285	9.39 mW	64	601.0 mJ/cm²	6.8	7
310	9.99 mW	60	599.4 mJ/cm²	6.8	7
365	9.87 mW	61	602.1 mJ/cm²	6.8	7
448	9.56 mW	63	602.3 mJ/cm²	6.8	7

10 min, dried at a critical point, and finally ion sputter coated. Morphological changes were observed with scanning electron microscope (SEM; S-4500; Hitachi, Tokyo, Japan). The experiment was repeated twice independently.

Quantitative real-time polymerase chain reaction

P. gingivalis was irradiated at 310 nm and incubated for either 5, 30, 60, 120, or 240 min, 1, 2, 3, or 5 days. RNA extraction and reverse transcription were performed as previously described.[27] RNA quality was determined by RNA integrity number (RIN: 1–10 values), a robust and reproducible RNA integrity calculation algorithm, using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) with an RNA 600 Pico Chips (Agilent Technologies).

Quantitative real-time polymerase chain reaction was performed as previously described,[28] for either 5, 30, 60, 120, or 240 min. Primers were designed from the NCBI database entry for *P. gingivalis* strain ATCC 33277 (NC 010729) and are shown in Table 2. All data were analyzed based on the comparative CT method. The resulting relative values represented the relative expression level of a given gene compared with the level in the nonirradiated control at a corresponding incubation time point ($n=4$).

Cytotoxicity on HGF-1

HGF-1 (ATCC CRL-2014, Manassas, VA) were cultured in Dulbecco's modified Eagle medium (Wako, Osaka, Japan), supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA), and 1% antibiotic-antimycotic mixture (Invitrogen) in a humidified atmosphere with 5% carbon dioxide (CO_2) at 37°C. The cells were seeded into 96-well plates (10,000 cells/well). At 48 h after seeding, medium was replaced with PBS, and HGF-1 cells were irradiated by UV LEDs. Treatments with chemical agents [0.025% benzalkonium chloride (BKC) and 3% hydrogen peroxide (H_2O_2)] for 20 s were used as positive control groups. The viability of irradiated HGF-1 was measured using WST-8 assay (Cell Counting Kit-8; Dojindo, Kumamoto, Japan) according to the manufacturer's instructions at days 1 and 3 following irradiation. The ratio (%) of the cell viability of irradiated or treated HGF-1 relative to that of nonirradiated control was calculated ($n=3$).

Table 2. Polymerase Chain Reaction Primers

Gene ID	*Gene name*	*Direction*	*Primer sequence (5′-3′)*	*Size (bp)*	*Description*
PGN_0001	*dnaA*	Forward	TTTGGAGGGCAATTTCGTAG	124	Chromosome replication initiator
		Reverse	TGTCACCGTACCGGGATATT		
PGN_0041	*htpG*	Forward	AATGGAAAGACGGCAAGATG	91	Heat shock protein 90
		Reverse	TTGAGGTCAGCAGGCTTTTT		
PGN_0043	*ftsH*	Forward	GTAGGAGCCTCTCGTGTTCG	132	Cell division protein
		Reverse	CTCATCATTGCCGGAGAAA		
PGN_0631	*ftsZ*	Forward	TACCACCGATCCGGAGTTAG	111	Cell division protein
		Reverse	ACATTGCCAAGGTTGTCTCC		
PGN_1057	*recA*	Forward	GAATGGCCACGGAGAAGATA	137	DNA repair
		Reverse	GTAACCGCCTACACCGAGAG		
PGN_1126	*(UmuD)*	Forward	AGACAAGTCCCTTGAAGCGC	115	Error-prone repair: SOS-response transcriptional repressor UmuD homolog
		Reverse	GTTGGCCGGTTCGAGTAGAA		
PGN_1127	*(UmuC)*	Forward	TGGTTGTATCATCGCTCGCA	101	SOS mutagenesis and repair protein UmuC homolog
		Reverse	CCACATTGTGCCGACGAATC		
PGN_1208	*clpB*	Forward	AGAAACTGCCCCATGTATCG	129	ClpB protein
		Reverse	CTAGCACGATGTGCTCCAAA		
PGN_1451	*groES*	Forward	ATTCGGCCAAAGAGAAACCT	95	Chaperonin GroES
		Reverse	TACGGTGTCTCCTGCTTTGA		
PGN_1452	*groEL*	Forward	TAGAATTGGAGTGCCCGTTC	153	Chaperonin GroEL
		Reverse	CTCCTGCCGTAACGTTCTTC		
PGN_1786		Forward	GAGCCAAGCGCGTATCTATC	118	DNA polymerase III β chain
		Reverse	TCTTCAGCAGCCACAGAGAA		

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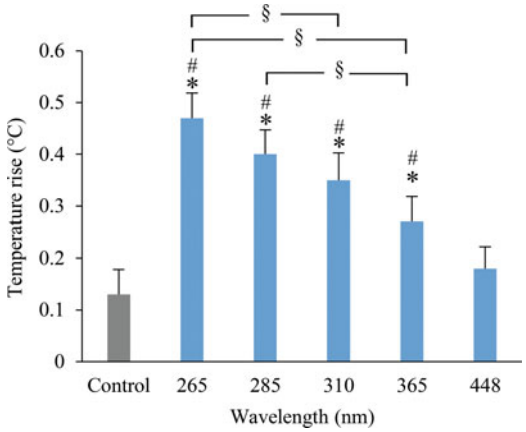

FIG. 2. Temperature change of *Porphyromonas gingivalis* suspension after UV irradiations. Data are presented as the mean ± SD ($n = 10$). $^{*}p < 0.05$ compared to nonirradiated control group. $^{\#}p < 0.05$ compared to 448 nm group. $^{\S}p < 0.05$. SD, standard deviation.

Statistical analysis

One-way analysis of variance (IBM® SPSS® Statistics 22) was used with Tukey's test as a *post hoc* test to compare differences among the groups, for all statistical analyses. A p value of <0.05 was considered significant.

Results

Temperature elevation of P. gingivalis *bacterial suspension after UV irradiations*

The temperature elevations of *P. gingivalis* bacterial suspensions after UV irradiations were very low, under 0.5°C, for all wavelengths with a significant difference compared with control ($p < 0.05$), except for 448 nm. The shortest UV wavelength 265 nm produced the highest temperature elevation (Fig. 2).

CFUs of UV-irradiated bacteria

No bacterial colonies were observed in the groups receiving 265 or 285 nm irradiation. Approximately 1 log reduction of CFUs were observed in the 310 nm irradiated group compared with the control group ($p < 0.05$; 1.02 log reduction for *P. gingivalis*, 0.74 for *P. intermedia*, 1.27 for *F. nucleatum*, 1.59 for *A. actinomycetemcomitans*, and 1.26 for *S. oralis*). The number of CFUs for both 365 and 448 nm did not differ from those of controls for any bacteria (Fig. 3).

P. gingivalis *growth (OD changes) following UV irradiation*

P. gingivalis irradiated at 265 or 285 nm showed little growth during 24 h, while a slight growth increase was

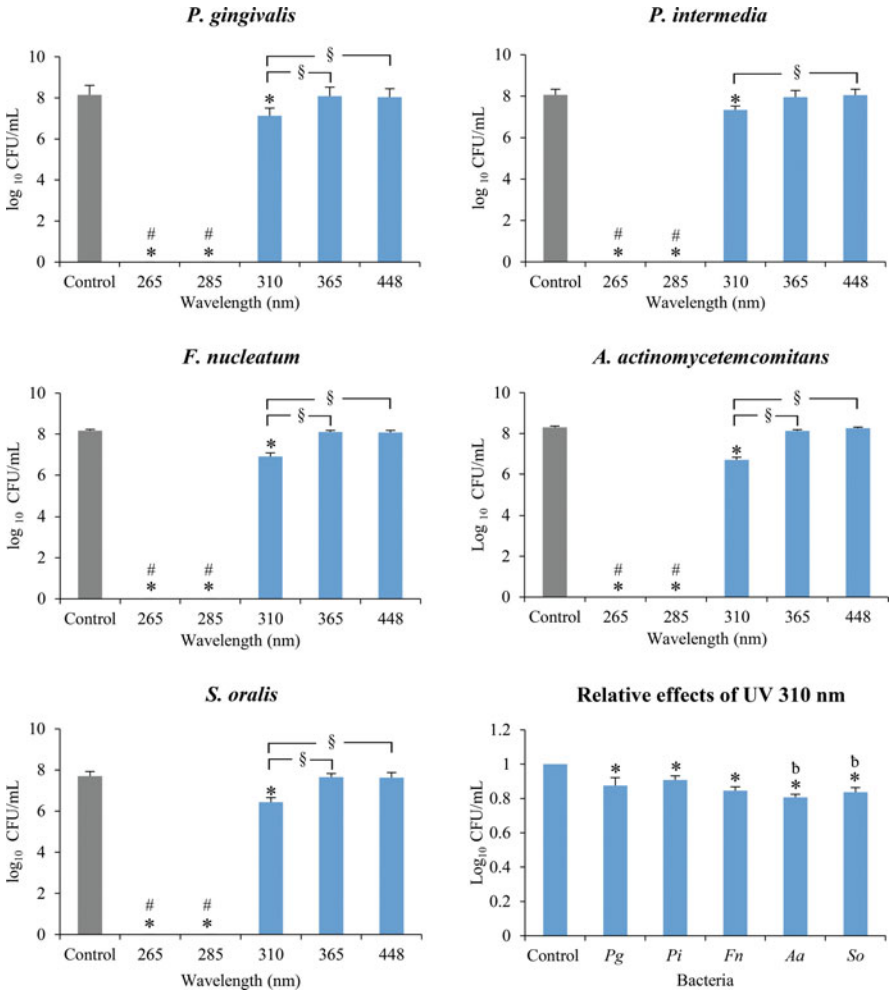

FIG. 3. CFUs following UV LED irradiations at various wavelengths on periodontopathic and oral bacterial suspensions. Data are presented as the mean ± SD ($n = 4$). $^{*}p < 0.05$ compared to nonirradiated control group. $^{\#}p < 0.05$ compared to 310, 365, and 448 nm groups. $^{b}p < 0.05$ compared to *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum* groups. $^{\S}p < 0.05$. CFUs, colony forming units; SD, standard deviation.

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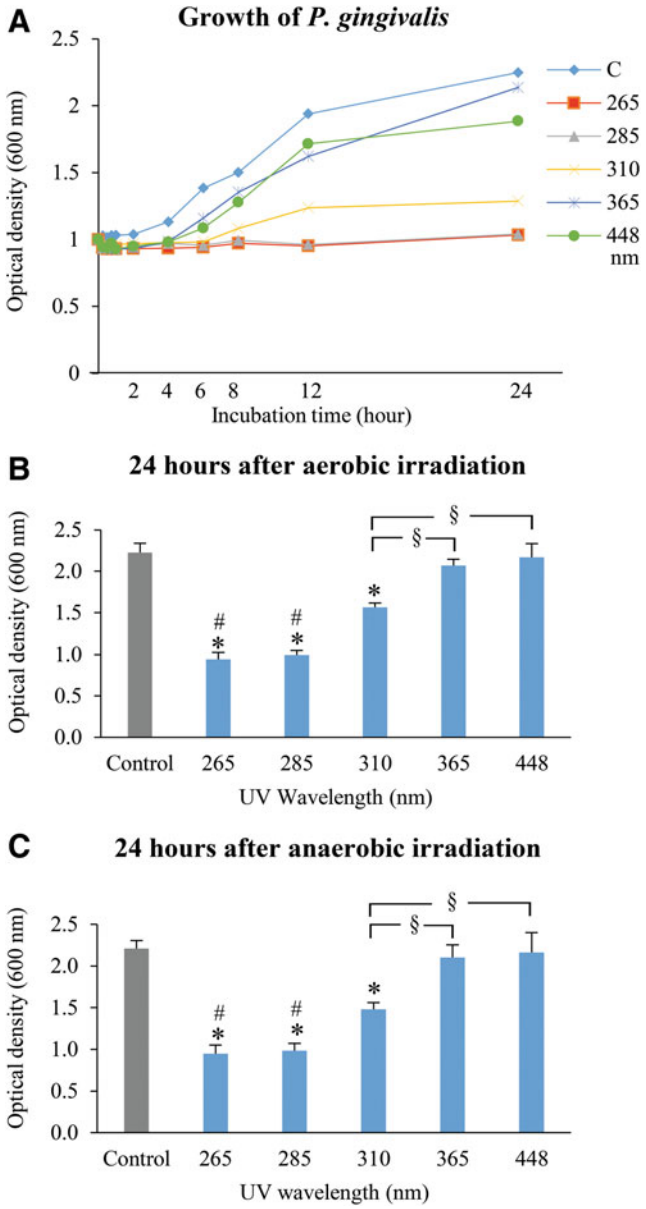

FIG. 4. *Porphyromonas gingivalis* growth in culture medium. Bacterial growth was indicated as an optical density (OD_{600}). **(A)** *P. gingivalis* growth curves after anaerobic UV LED irradiations, C: control. **(B)** *P. gingivalis* growth at 24 h after aerobic irradiation. **(C)** *P. gingivalis* growth at 24 h after anaerobic irradiation. Data are presented as the mean ± SD ($n = 3$). $^{*}p < 0.05$ compared to nonirradiated control group. $^{\#}p < 0.05$ compared to 310, 365, and 448 nm groups. $^{\S}p < 0.05$. SD, standard deviation.

observed with 310 nm irradiation. *P. gingivalis* showed no difference in growth among 365 nm, 448 nm, and control groups (Fig. 4A). For irradiations under aerobic and anaerobic conditions, *P. gingivalis* growth showed similar growth trends (Fig. 4B, C).

SEM analysis of UV-irradiated P. gingivalis

There were no morphological changes of *P. gingivalis* in all UV-treated groups after 30 min or 1 day following irradiation. Morphological alterations with irregular and deformed cells were observed from day 2 postirradiation for 265 and 285 nm, and from day 3 for 310 nm. Boundaries of adjacent bacterial cells became blurred at that time. Five days after irradiation, *P. gingivalis* cells have no trace of their original forms and appeared completely broken and fragmented for 265, 285, and largely broken for 310 nm groups, while there were no notable changes of bacterial cell forms in 365 and 448 nm groups for a period of 5 days postirradiation (Fig. 5).

RNA quality and gene expression profiling in P. gingivalis *following 310 nm irradiation*

RIN value showed little change (around 8.0–9.1) up to 4 h following irradiation. However, the RIN value decreased substantially to under 2.5 on days 1, 2, 3, and 5.

At 30–60 min, gene expressions related to chaperones [*htp*G (PGN_0041), *clp*B (PGN_1208), *GroES* (PGN_1451), and *GroEL* (PGN_1452)] were remarkably increased and then gradually decreased. Whereas, SOS response-related genes [*UmuD* (PGN_1126), *UmuC* (PGN_1127), *RecA* (PGN_1057), and DNA polymerase III (PGN_1786)], and genes related to chromosome replication (PGN_0001), and cell division (PGN_0043), were inhibited more than twofold at 30-min postirradiation followed by a gradual recovery (Fig. 6).

Cytotoxicity of UV irradiations on HGF-1

Approximately 60 s irradiation at 265 or 285 nm completely devitalized HGF-1 on days 1 and 3. Also, 20 s treatment of H_2O_2 or BKC showed complete devitalization. 310 nm reduced viability by ~30% and 50% at days 1 and 3, respectively, whereas 365 and 448 nm resulted in no significant reduction compared with control group (Fig. 7).

Discussion

In this study, the bacteria were exposed to a total ED of ~600 mJ/cm^2 with a portable UV LED device *in vitro*. Temperature elevations of the bacterial solutions after irradiation were negligible at all wavelengths, and there should be no noteworthy influence from thermal effects induced by UV LED irradiation on bacterial viability. This nonthermal devitalization of bacteria may be one of the advantages of using UV LEDs, for bacterial reduction during periodontal treatment. It does not produce thermal damage to the surrounding periodontal tissues. This is in contrast to thermal devitalization/evaporation of bacteria using relatively high power lasers, such as CO_2, Nd:YAG, Diode, and Er:YAG lasers, which results in thermal damage to host tissues and cells.

Interestingly, with decreasing wavelength, the temperature-elevation increased proportionally, suggesting greater bacterial absorption of shorter wavelengths that may be related to the increased bactericidal effects of shorter wavelengths such as 265, 285, and 310 nm. In fact, a previous study showed that among UV LED wavelengths, UVC light ~254 nm is most readily absorbed by DNA, resulting in the strongest bactericidal effects.[29] Consequently, 1-week postirradiation, 265 and 285 nm completely suppressed CFU formation and 310 nm produced a significant 1 log reduction

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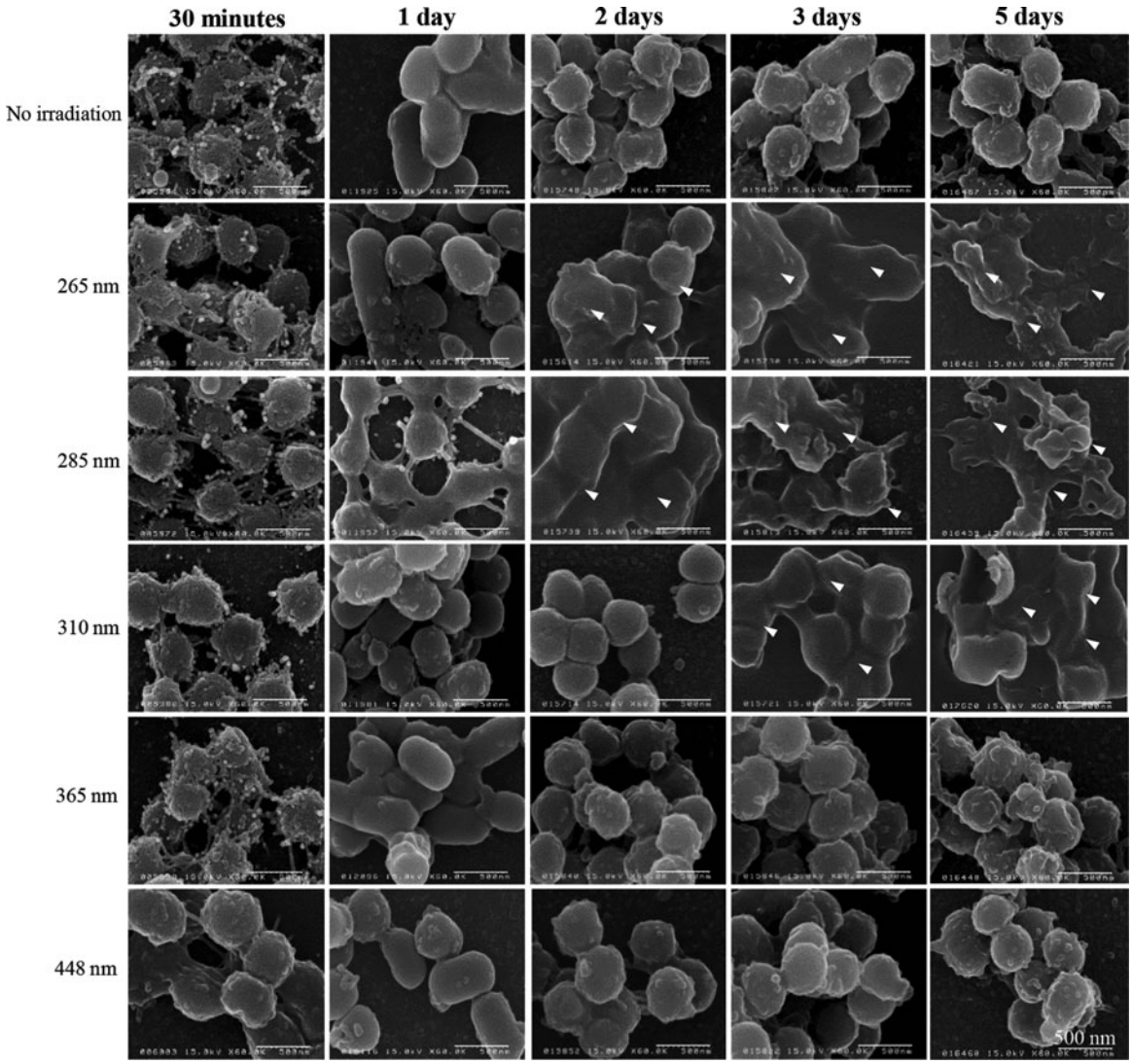

FIG. 5. Scanning electron microscope micrographs of *Porphyromonas gingivalis* following UV LED irradiation at various wavelengths. White arrowhead: alteration of bacterial cell morphology.

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(90% killing) of CFUs for all bacteria examined. Thus, 265 and 285 nm irradiations revealed strong and complete bactericidal effects, whereas 310 nm showed weaker bactericidal and growth suppression effects on bacteria.

P. gingivalis, following 265 and 285 nm irradiations, did not grow up to 24 h (no OD increase or decrease during that period). This was in accord with SEM findings of no destruction of *P. gingivalis* on day 1 for 265 and 285 nm. Further, following 310 nm irradiation, OD increased, to a lesser extent, indicating partial growth suppression. Interestingly, results were similar under both aerobic and anaerobic irradiation conditions, suggesting no influence by the presence of oxygen during irradiation.

With SEM analysis, initial alteration of bacterial cell morphology was observed around day 2 for 265 and 285 nm, and around day 3 for 310 nm. Severe destruction was observed on day 5 for these wavelengths. At the same time, according to the RIN value measurement, the total RNA from *P. gingivalis* irradiated at 310 nm showed no destruction until 4 h; however, the RNA severely degraded on days 1–5. Thus, it appears that UV wavelengths may not immediately devitalize bacteria by directly affecting bacterial cell walls. Previous studies described that shorter wavelength UVC irradiation can induce DNA pyrimidine dimer lesions that in turn inhibit cell proliferation and apoptosis, eventually leading to cell death.[30,31]

In contrast, a-PDT, which produces reactive oxygen species, shows a different mechanism to destroy bacteria. We have previously demonstrated that a-PDT with combined rose Bengal and blue LEDs immediately reduced OD reaching a minimal plateau after 40 min, suggesting the induction of immediate destruction of cells. Almost complete degradation of RNA has also been observed 3 h post-treatment.[32]

Bacteria must survive challenging conditions to colonize or invade host cells. These stresses can cause cellular proteins to denature and form insoluble aggregates that are devoid of biological activity.[33] Bacterial cells produce stress proteins including molecular chaperones. They help prevent, or manage, the misfolding and aggregation of proteins that

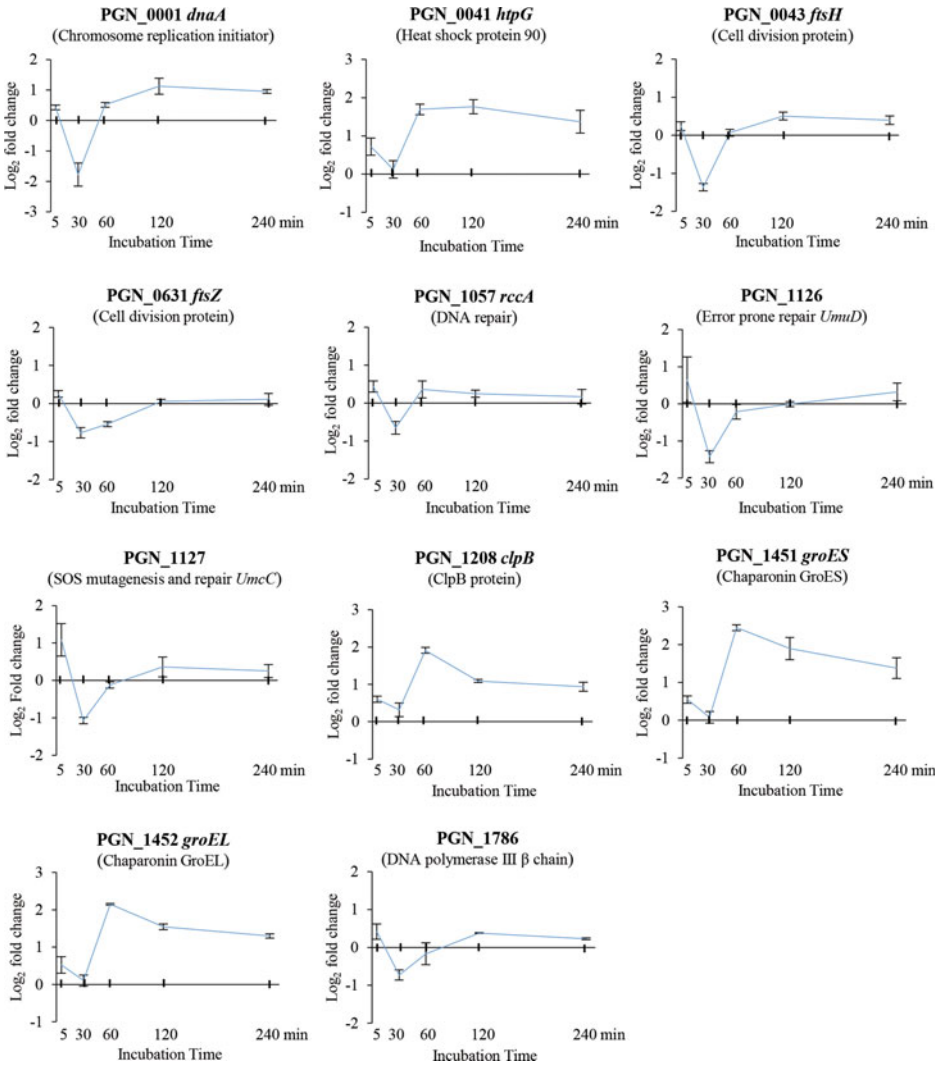

FIG. 6. Gene expression profiling of *Porphyromonas gingivalis* irradiated at 310 nm compared to nonirradiated control. The fold change [$\log_2$ fold change (vs. control)] in gene expression at each time point was calculated based on the comparative CT method. Data are presented as the mean ± SD ($n = 4$). SD, standard deviation.

are threats to all living organisms. In this study, following irradiation, chaperone genes (*GroEL*, *GroES*, *HtpG*, and *ClpB*) were rapidly upregulated after 30 min. This suggests that 310 nm UV irradiation may induce a broad stress response in *P. gingivalis*.[34,35] In addition, the bacteria surviving following irradiation (~10%) may have suffered nonfatal DNA damage affecting genes associated with metabolism and bacterial cell growth. Their chaperon gene expression levels remained relatively high after 60 min, suggesting that chaperone proteins may be providing support to damaged proteins.

SOS response in bacteria is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis are induced.[36] Most mutagenesis resulting from exposure to UV radiation requires the operation of a specialized system involving the *UmuD*, *UmuC*, *RecA*, and DNA polymerase III proteins, which allows translesion synthesis to occur on damaged DNA templates.[37] In our study, SOS response genes showed similar gene expression profilings, following a rapid decrease from 5 to 30 min and then a restoration to the control level, suggesting that 310 nm UV irradiation may inhibit gene expressions and induce a decrease in cell number at stationary phase. That is also supported by findings of similar expression profiles among DNA replication initiator (*DnaA*) and cell division protein (*FtsH* and *FtsZ*). In our previous study, blue LED (425–500 nm) did not degrade RNA but inhibited *P. gingivalis* growth by suppressing DNA replication and cell division-related genes.[27]

According to SEM and RNA findings with 310 nm irradiation, it took a relatively short time for the UV-irradiated bacterial cells to change gene expressions and a relatively long time to change cell morphology. This suggests that the most UV-irradiated bacteria may undergo chromosome inactivation, transcriptional suppressions, and production of defective proteins at least within 4 h. Then, the bacterial cell may begin to loose activity within 24 h. However, even after devitalization, cell structure will not be destroyed for up to 48 h. Some surviving bacterial cells may avoid the mutation of genes indispensable to life support, and instead, only transiently experience cell stress and growth suppression.

Finally, to evaluate phototoxicity of UV wavelengths to periodontal tissue, the effects on experimentally prepared HGF-1 cells were examined, as compared to a nonirradiated control and oral antiseptic positive controls (BKC and H_2O_2). Interestingly, the following irradiation effects on HGF-1 relative cell viability correlated with the irradiation effects on bacterial cell death. Both 265 and 285 nm

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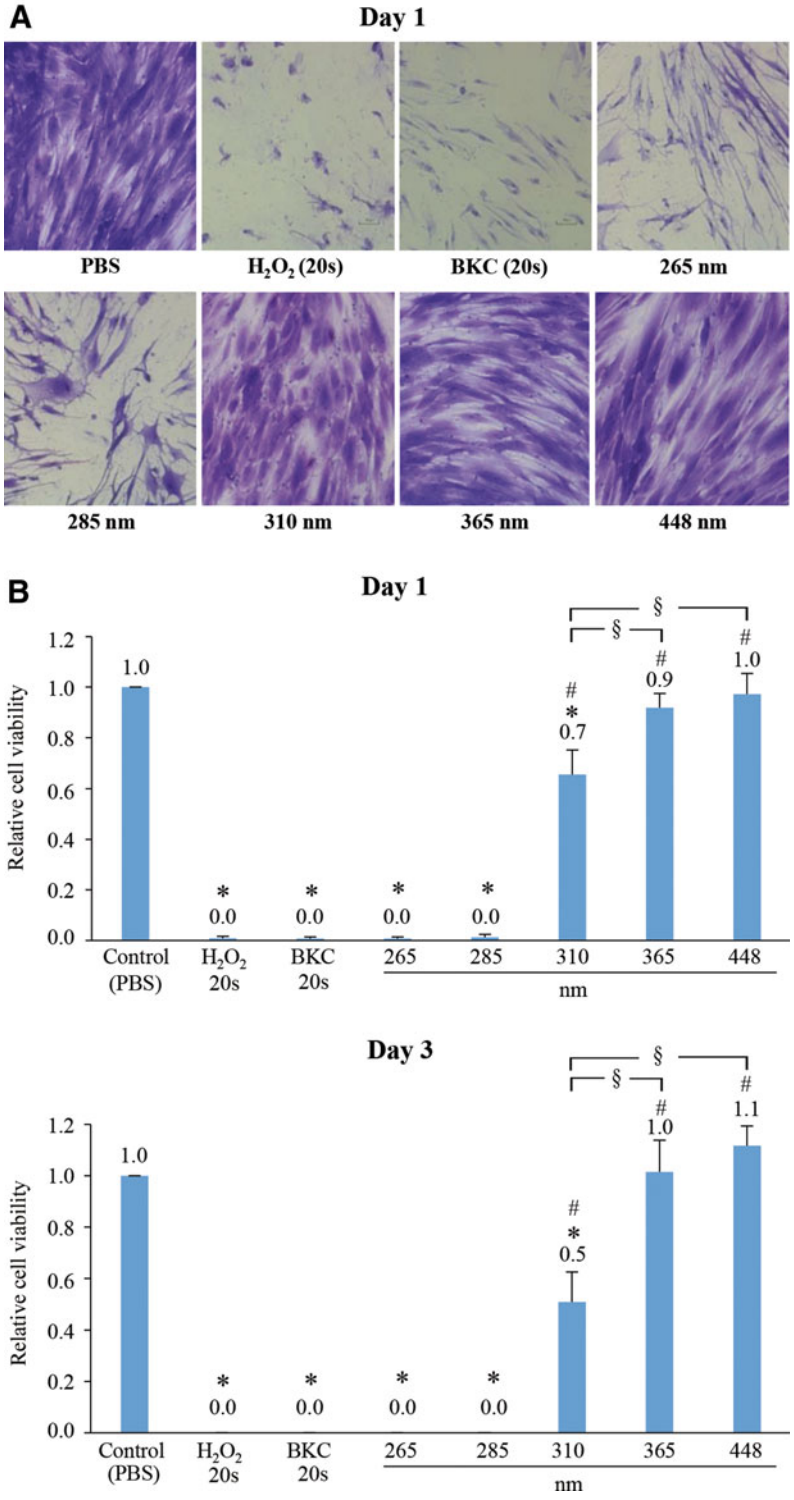

FIG. 7. **(A)** Crystal violet staining images of human gingival fibroblasts at day 1 after UV LED irradiation. **(B)** Viability of human gingival fibroblasts at days 1 and 3 after UV LED irradiations. Oral antiseptic agents [3% H_2O_2 (20 s) and 0.025% BKC (20 s)] were used as cell-damaging controls. Data are presented as the mean ± SD ($n = 3$). $^{*}p < 0.05$ compared to nonirradiated control group. $^{\#}p < 0.05$ compared to H_2O_2, BKC, 265, and 285 nm groups. $^{\S}p < 0.05$. BKC, benzalkonium chloride; H_2O_2, hydrogen peroxide; PBS, phosphate-buffered saline; SD, standard deviation; UV LED, ultraviolet light-emitting diode.

irradiations, and treatment with oral antiseptic agents (BKC and H_2O_2) for 20 s, severely damaged and devitalized the fibroblasts. In contrast, 310 nm irradiation showed much less harmful effects on the cells.

Since 310 nm irradiation has already been used to treat skin diseases in medicine, our findings with regard to the reduced phototoxicity of 310 nm irradiation support the possibility that it might also be effective for bacterial growth inhibition in periodontal therapy while posing less risk to the surrounding tissues. Even though 265 and 285 nm UV LED lights are clearly cytotoxic to human host cells, their strong bactericidal effects may be useful for disinfection of surgically exposed/isolated tooth or implant surfaces. While these results obtained from *in vitro* study do not necessarily ensure efficacy in clinical situations, we consider that UVC and UVB LED irradiations have the possibility for use as new means of periodontal and/or peri-implant therapy. We plan to pursue further studies regarding clinical applications.

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Conclusions

Under present irradiation conditions, both 265 and 285 nm may induce complete bactericidal effects, as well as complete death of irradiated gingival fibroblasts, *in vitro*; whereas 310 nm achieves partial killing and inhibition of bacterial growth with much less phototoxicity to fibroblasts. This suggests that UVC and UVB light with shorter wavelengths may have potential to be applied for bacterial suppression in periodontal and peri-implant therapy. Such UV light use may have different applications in various clinical situations depending on the wavelength.

Acknowledgments

We thank Dr. Kenji Matsushita, National Center for Geriatrics and Gerontology, and Drs. Shizuko Ichinose, Yuriko Sakamaki, Yujin Ohsugi, and Yutaro Kitanaka, TMDU for their advice and help. Support was provided by the Research Funding for Longevity Sciences (29-3) program administered by the National Center for Geriatrics and Gerontology.

Author Disclosure Statement

No competing financial interests exist.

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Received: June 20, 2018
Accepted after revision: November 22, 2018
Published online: April 5, 2019.

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